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Adaptive strategies by Southern Ocean phytoplankton to lessen iron limitation: Uptake of organically complexed iron and reduced cellular iron requirements

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Abstract

We report results of laboratory studies examining the effect of low levels of iron (Fe) availability on the intracellular Fe concentrations and specific growth rates in Southern Ocean diatoms (*Fragilariopsis kerguelensis*, *Eucampia antarctica*, *Proboscia inermis*, and *Thalassiosira antarctica*) and *Phaeocystis antarctica*. All species grew on Fe complexed to the siderophore desferrioxamine B (DFB). Concentrations of DFB up to 100-fold in excess of Fe were required to limit growth rates by ≥ 50%. Southern Ocean phytoplankton also grew on Fe complexed by ≥ 10-fold excess concentrations of the siderophores ferrichrome, enterobactin, or aerobactin, whereas the temperate coastal diatoms *Thalassiosira weissflogii* and *Thalassiosira pseudonana* did not. Intracellular Fe concentrations and Fe : C ratios of all Southern Ocean species were exceptionally low and decreased with decreasing Fe availability. However, large diatoms had significantly lower cell-volume–normalized Fe content and Fe : C ratios than *Phaeocystis*. Short-term Fe uptake and extracellular Fe(II) production measurements provided evidence that *Phaeocystis* possesses a reductive Fe transport pathway. Our findings demonstrate that the large-diatom Fe requirements are at least 2-fold lower than currently reported for oceanic algal species and suggest that bioreduction may enable resident phytoplankton to directly use Fe bound to strong organic ligands.

Over the last 15 yr, numerous field and laboratory bioassay experiments (Martin et al. 1990), and a series of in situ mesoscale iron- (Fe) enrichment experiments (Boyd et al. 2007), have provided compelling evidence that Fe availability both limits phytoplankton metabolic processes and influences phytoplankton community structure in large regions of the ocean, the largest of which is the Southern Ocean. These subpolar and polar waters are geographically isolated from arid regions and so are characterized by low external Fe inputs from dust deposition (Wagener et al. 2008) that contribute to the very low concentrations of dissolved Fe in the upper ocean (~ 0.07 nmol L^{-1}; Measures and Vink 2001).

The Southern Ocean is thought to have a disproportionate influence, via both ocean circulation and biogeochemistry, on the global carbon (C) cycle (Sarmiento et al. 1998). Changes in the supply of Fe to the oceans in the geological past are also evident, and are thought to have played a partial role in mediating atmospheric CO_2 levels by elevating rates of primary and export production (Sigman and Boyle 2000). This importance of Fe availability in setting the rate of phytoplankton growth, and both primary and export production in the Southern Ocean, has resulted in considerable research into the mode and magnitude of Fe supply in these waters in the present day and how these might alter in the future (Boyd and Ellwood 2010).

Despite this focus on Fe supply mechanisms and their relative contribution to Southern Ocean waters, including a synthesis of the recent advances (Moore and Braucher 2008), there has been less integration regarding the 'flip side' of Fe supply—phytoplankton Fe acquisition rates and cellular demand. For example, bloom-forming Southern Ocean phytoplankton are reported to act as major vectors for particulate organic C export into the oceans interior driven by both *Phaeocystis* (DiTullio et al. 2000) and diatom (Buesseler et al. 2001) species, yet reported Fe requirements for these groups vary widely. Phytoplankton Fe requirements are typically assessed by determining the external concentration of Fe required to support growth or by estimating or measuring their intracellular or biochemical Fe content. In the former case, phytoplankton growth is measured over a range of dissolved (Fe_{diss}) or inorganic (Fe′) Fe concentrations and the half-saturation constant for growth (K_m) calculated. Using this approach, Coale et al. (2003) reported a wide range of K_m values for diatoms (0.007–0.125 nmol L^{-1} Fe_{diss}) and *Phaeocystis* (0.005–0.258 nmol L^{-1} Fe_{diss}) in Ross Sea and Antarctic Circumpolar Current waters. An even greater range in K_m values (0.0006–1.14 nmol L^{-1} Fe_{diss}) has been reported for lab-cultured Southern Ocean diatoms by Timmermans et al. (2001, 2004), who suggested that K_m values increase with increasing cell size. While these studies emphasize the role of dissolved Fe in supplying cellular demand, it has become apparent that the majority of dissolved Fe in the Southern Ocean is bound to organic ligands that affect its chemical speciation (Boye et al. 2001). Understanding the potential availability of such complexed Fe to marine phytoplankton

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requires knowledge of the physiological mechanism of Fe uptake. A central question then is what chemical forms of dissolved Fe are available for biological uptake?

To date, very few studies have examined the cellular Fe requirements of phytoplankton isolated from Fe-limited oceanic regions. We are aware of only one laboratory study that has measured the steady-state cellular Fe content of Southern Ocean isolates (Lane et al. 2009). This study found that the large diatom *Proboscia inermis* has an exceptionally low cellular Fe : C ratio (0.67 μmol mol^{-1}) compared to oceanic and coastal species from temperate environments (Sunda and Huntsman 1995, 1997). Similarly, Maldonado and Price (1996) reported a comparably low Fe : C ratio for a large diatom *Thalassiosira subtilis* isolated from the Fe-limited equatorial Pacific (0.65–1.7 $\pm$ 0.1 μmol mol^{-1}). In contrast, Timmermans et al. (2001) have suggested that the cellular Fe demands of some Southern Ocean diatoms exceed those of both oceanic and coastal diatoms from temperate regions, with low and high Fe requirements respectively, and that cellular Fe requirements increase with cell size. However, the field measurements of Twining et al. (2004) using single-cell elemental mapping reveal an inverse relationship between Fe : C and cell size. Such an inverse relationship between Fe : C and cell size is also apparent in the data sets of Sunda and Huntsman (1995, 1997) for temperate oceanic and coastal phytoplankton from non–Fe-limited regions.

This evidence of such a wide range of cellular Fe requirements begs the question—is this wide range of Fe requirements driven by interspecific, regional and/or methodological differences? How much of this range can be explained by experimental artifact vs. the ability of diatoms and other phytoplankton isolated from Fe-impoverished environments to phenotypically acclimate to Fe limitation, or of the genetic adaptations that are likely to have arisen from the selective pressure imposed by low Fe supply?

The first objective of this study was to test the ability of two dominant bloom-forming groups of Southern Ocean phytoplankton, diatoms and the haptophyte *Phaeocystis antarctica*, to access and, hence, grow on organically complexed Fe. We used terrestrial hydroxamate (desferrioxamine B [DFB] and ferrichrome) and catecholate (enterobactin) siderophores as models of marine siderophores with similar Fe-binding functional groups, and the marine siderophore, aerobactin, with mixed α-hydroxycarboxylate and hydroxamate functional groups. The second objective of this study was to test the hypothesis that large cells (i.e., diatoms) have higher Fe requirements. To accomplish this goal, we freshly isolated Southern Ocean phytoplankton (ranging over three orders of magnitude in cell volume) that have been observed to bloom upon purposeful or natural Fe fertilization of Southern Ocean waters, including *Fragilariopsis kerguelensis*, *Eucampia antarctica*, *Proboscia inermis*, and *Thalassiosira antarctica*. Here we provide the first systematic investigation of the intracellular Fe concentrations of these organisms grown under controlled steady-state conditions of Fe limitation.

Methods

Study organisms—We isolated four diatoms and the haptophyte *Phaeocystis antarctica* from water collected south of the Polar Frontal Zone in December 2001. The centric diatoms *Proboscia inermis*, *Eucampia antarctica*, *Thalassiosira antarctica*, and the pennate diatom *Fragilariopsis kerguelensis* were sampled from the Australasian sector of the Southern Ocean at 57°51.1′S and 139°50.7′E. *P. antarctica* (clone AA1) was isolated at 61°20.8′S, 139°50.6′E. Stable stock cultures were established by July 2002. An additional *P. antarctica* strain (clone SX9) was isolated in December 2004 from oceanic waters (65°08.72′S and 174°08.94′E). Both *P. antarctica* clones were isolated from a single colony. However, after $\sim$ 3 yr in culture, clone AA1 stopped forming colonies. Therefore, all results reported for this strain are for solitary cells. Clone SX9 retained the ability to form colonies at the time of sampling. Both *Phaeocystis* strains were rendered axenic by treatment with antibiotics, which was confirmed by epifluorescence microscopy on cultures treated with 4′,6-diamidino-2-phenylindole (DAPI). Southern Ocean diatom cultures were not axenic. However, bacterial abundances were monitored prior to harvest, with bacteria contributing $< 0.1\%$ to total C, assuming a conversion factor of 12.4 fg C cell^{-1} (Fukuda et al. 1998).

In several experiments we also examined three temperate diatoms of the genus *Thalassiosira*: *T. oceanica* (clone 1003), an oceanic isolate from the Sargasso Sea, and the coastal isolates *T. pseudonana* (clone 3H, Moriches Bay, Long Island, New York) and *T. weissflogii* (clone Actin, Long Island Sound, New York). All were obtained from the Provasoli–Guillard Center for Culture of Marine Phytoplankton (West Boothbay Harbor, Maine) and maintained under axenic conditions.

Medium preparation—All phytoplankton cultures were grown in the artificial seawater medium Aquil (Price et al. 1988/1989), enriched with 10 μmol L^{-1} phosphate, 100 μmol L^{-1} silicate, 300 μmol L^{-1} nitrate, and filter-sterilized (0.2 μm Gelman Acrodisc PF) trace-metal and vitamin solutions (Maldonado and Price 1996). Free trace-metal ion concentrations, in the presence of 10 μmol L^{-1} ethylenediaminetetraacetic acid (EDTA) as the chelating agent, were as follows ($-$log free-metal ion concentration = pMetal): pCu 14.07, pMn 8.17, pZn 10.79, and pCo 11.09. These concentrations were calculated using the chemical equilibrium computer program Visual MINTEQ (http://www.lwr.kth.se/English/OurSoftware/vminteq/) for Aquil media at 3°C and the initial pH of the medium (8.17 $\pm$ 0.04 [n = 13]). Selenite and molybdate were added at 10^{-8} and 10^{-7} mol L^{-1} respectively. The salinity of the medium was 35.

The basal Aquil medium contained 1.8 $\pm$ 0.1 nmol L^{-1} Fe contamination (n = 6), measured using electrochemical techniques following Tian et al. (2006). All Southern Ocean strains (exception for *F. kerguelensis*) maintained maximum growth rates in Aquil media that contained no added Fe and either 10 μmol L^{-1} or 100 μmol L^{-1} of EDTA (Table 1). We, therefore, assumed that a significant fraction of the Fe contamination was bioavailable and it

Table 1. Growth rates (μ [d^{-1}]) of Southern Ocean phytoplankton grown in synthetic and natural seawater (SASW) media. Iron-replete and Fe-limiting treatments used in subsequent experiments are highlighted in boldface. 'ng' indicates the cultures did not grow.

Iron treatment	[Fe′] (pmol L^{-1})*	μ (d^{-1})	SE	*n*	$\mu : \mu_{max}$
Phaeocystis antarctica (clone AA1)					
58 nmol L^{-1} 10 μmol L^{-1} EDTA	3450	0.52	0.02	12	1.00
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	**258**	**0.52**	**0.02**	**36**	**1.00**
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.51	0.03	12	0.97
1.8 nmol L^{-1} 10 μmol L^{-1} EDTA	106	0.47	0.00	9	0.90
1.8 nmol L^{-1} 100 μmol L^{-1} EDTA	10.6	0.47	0.00	6	0.90
0.8 nmol L^{-1} 10 μmol L^{-1} EDTA	47.1	0.53	0.03	3	1.02
FeDFB† 2 : 2 (nmol L^{-1} : nmol L^{-1})	14.3	0.53	0.02	6	1.02
FeDFB 4 : 4	30.1	0.50	0.02	18	0.96
FeDFB 4 : 40	0.17	0.50	0.02	12	0.97
FeDFB 2 : 200	0.01	0.30	0.01	6	0.58
FeDFB 4 : 400	**0.02**	**0.28**	**0.04**	**39**	**0.54**
FeDFB 4 : 4000	0.01	ng	—	3	0.00
SASW (0.49 nmol L^{-1} Fe, 1.4 nmol L^{-1} ligand)	4.23	0.51	0.06	12	0.98
Fragilariopsis kerguelensis					
58 nmol L^{-1} 10 μmol L^{-1} EDTA	3450	0.32	0.01	17	1.00
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	258	0.18	0.01	9	0.56
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.15	0.02	6	0.48
1.8 nmol L^{-1} 10 μmol L^{-1} EDTA	106	0.13	0.02	6	0.41
FeDFB 4 : 4	30.1	0.15	0.01	12	0.46
FeDFB 4 : 40	0.17	0.15	0.01	10	0.47
FeDFB 4 : 400	0.02	0.08	0.03	3	0.25
Thalassiosira antarctica					
58 nmol L^{-1} 10 μmol L^{-1} EDTA	3450	0.12	0.02	3	1.00
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.15	0.02	3	1.28
1.8 nmol L^{-1} 10 μmol L^{-1} EDTA	106	0.14	0.01	3	1.23
FeDFB 4 : 4	30.1	0.09	0.01	3	0.79
FeDFB 4 : 40	0.17	0.05	0.03	3	0.42
FeDFB 4 : 400	0.02	0.04	0.01	3	0.34
Eucampia antarctica					
58 nmol L^{-1} 10 μmol L^{-1} EDTA	3450	0.36	0.01	6	1.00
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	**258**	**0.34**	**0.02**	**8**	**0.93**
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.36	0.02	3	1.00
1.8 nmol L^{-1} 10 μmol L^{-1} EDTA	106	0.33	0.02	3	0.92
FeDFB 4 : 4	30.1	0.23	0.01	3	0.64
FeDFB 4 : 40	**0.17**	**0.19**	**0.01**	**9**	**0.54**
FeDFB 4 : 400	0.02	ng	—	3	—
SASW (0.49 nmol L^{-1} Fe, 1.4 nmol L^{-1} ligand)	4.23	ng	—	3	—
Proboscia inermis					
58 nmol L^{-1} 10 μmol L^{-1} EDTA	3450	0.47	0.01	27	1.00
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	**258**	**0.47**	**0.01**	**48**	**1.00**
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.44	0.01	12	0.94
1.8 nmol L^{-1} 10 μmol L^{-1} EDTA	106	0.45	0.01	6	0.96
1.8 nmol L^{-1} 100 μmol L^{-1} EDTA	10.6	0.44	0.01	6	0.95
0.8 nmol L^{-1} 10 μmol L^{-1} EDTA	47.1	0.44	0.03	3	0.94
FeDFB 4 : 4	30.1	0.42	0.04	12	0.90
FeDFB 4 : 40	0.17	0.42	0.02	12	0.90
FeDFB 4 : 80	0.08	0.46	0.01	6	0.99
FeDFB 4 : 200	**0.03**	**0.29**	**0.02**	**18**	**0.61**
FeDFB 4 : 400	0.02	0.18	0.00	3	0.38
SASW (0.49 nmol L^{-1} Fe, 1.4 nmol L^{-1} ligand)	4.23	0.32	0.04	5	0.67

* The [Fe′] in Aquil medium containing 58 nmol L^{-1} Fe and 10 μmol L^{-1} ethylenediaminetetraacetic acid (EDTA) exceeds the empirically observed threshold for precipitation of Fe hydroxides ($\sim$ 700 pmol L^{-1}; Sunda and Huntsman 1995) and is, therefore, an overestimate.

† DFB = desferrioxamine B.

was, therefore, included in the calculation of total Fe. In one experiment, we grew *P. antarctica* (clone AA1) and *P. inermis* in Aquil treated with 8-hydroxyquinoline resin (Sigma–Aldrich) in place of Chelex 100, which reduced Fe contamination to 0.8 ± 0.1 nmol L^{-1}.

We also grew *E. antarctica*, *P. inermis*, and *P. antarctica* in filtered sub-Antarctic surface seawater (SASW; 46°14.4′S 175°48.9′E) collected using a trace-metal clean system (Tian et al. 2006). SASW was supplemented with chelexed macronutrients (10 μmol L^{-1} phosphate, 100 μmol L^{-1} silicate, and 300 μmol L^{-1} nitrate). No other additions were made. SASW was filter-sterilized (0.2-μm 47-mm polycarbonate filters; Poretics ®) before use. After all manipulations, SASW contained 0.49 ± 0.05 nmol L^{-1} Fe and 1.4 ± 0.1 nmol L^{-1} of natural organic ligands with a log conditional stability constant (log $K_{Fe'L}^{cond}$) of 11.1 ± 0.1 (n = 3), which was determined electrochemically.

Iron manipulation experiments—Southern Ocean cultures were grown at 3° ± 1°C under a continuous photon flux density (PFD) of 80–100 μmol quanta m^{-2} s^{-1}, which is saturating for all species examined (data not shown). PFDs were measured with a calibrated 4π quantum meter (model QSL-2101; Biospherical Instruments). Experimental cultures were inoculated using stock cultures grown in Aquil medium containing 2.2 nmol L^{-1} Fe and 10 μmol L^{-1} EDTA at < 10 μmol quanta m^{-2} s^{-1} on a 14 : 10 light : dark cycle. Experimental cultures were grown in triplicate and allowed to acclimate to growth conditions for at least two transfers (14–16 cell divisions) before data collection. Acclimated cultures were maintained in exponential phase by dilution. Growth rates of acclimated cells were determined from in vivo chlorophyll *a* fluorescence using a Turner Designs model 10-AU fluorometer. Specific growth rates (d^{-1}) were calculated from least-squares regressions of ln in vivo fluorescence vs. time during the exponential growth phase.

The Southern Ocean strains were grown in a variety of media that varied in their total Fe concentration, and in the ligand used to complex Fe. Iron-replete media were prepared by adding premixed, filter-sterilized FeEDTA (1 : 1.05) to Aquil containing 10 μmol L^{-1} of EDTA at three concentrations: 56.5, 2.6, and 0.6 nmol L^{-1}. Including Fe contamination, total Fe concentrations in the media were 58, 4.4, 2.4, 1.8 (no Fe added), and 0.8 (8-hydroxyquinoline–treated Aquil) nmol L^{-1}. Inorganic Fe concentrations ([Fe′]) were calculated for FeEDTA media following Sunda and Huntsman (2003) at the mean incubation temperature (3°C) and growth irradiance (90 μmol quanta m^{-2} s^{-1}), and at pH 8.3—the mean of the initial media (8.17 ± 0.04) and late-exponential phase cultures (8.4 ± 0.02). An overall conditional steady-state dissociation constant for Fe-EDTA chelates of 5.9×10^{-7} was used to calculate a $[Fe_{total}]$: [Fe′] ratio of 17.0 for Aquil media containing 10 μmol L^{-1} EDTA. The overall conditional dissociation constant was calculated as the sum of the conditional stability constant in the dark (2.0×10^{-7}) and the conditional photo-dissociation constant ($K_{hv} \times 0.18$; 3.9×10^{-7}) of EDTA at 3°C. K_{hv} at 3°C was estimated from values at 10°C and 20°C (Sunda and Huntsman 2003), assuming that K_{hv} is a linear function of temperature.

In order to induce Fe stress in the Southern Ocean strains, Fe was added complexed with the terrestrial siderophore desferrioxamine B mesylate (DFB; Sigma-Aldrich). We examined the growth of these strains in Aquil media to which DFB was added directly (no Fe added) or in which DFB was precomplexed to 2 nmol L^{-1} of $FeCl_3$ (in 0.01 mol L^{-1} quartz-distilled HCl) prior to addition. The Fe : DFB molar ratio in these media ranged from 1 : 1.05 to 1 : 1000. In most experiments, we added 2 nmol L^{-1} $FeCl_3$ complexed with 4, 40, 200, or 400 nmol L^{-1} of DFB to Aquil medium containing 10 μmol L^{-1} of EDTA to buffer the other trace metals. As we included the 1.8 nmol L^{-1} Fe contamination in the calculation of total Fe, the Fe : DFB ratios in these media were 3.8 : 4, 3.8 : 40, 3.8 : 200, and 3.8 : 400 nmol L^{-1}, corresponding to pFe values of 20.5, 22.8, 23.5, and 23.8, respectively. Hereafter, they are referred to as 4 : 4, 4 : 40, 4 : 200, and 4 : 400, respectively. [Fe′] in FeDFB media were calculated by the equation [Fe′] = [FeDFB] / ([L′] × $K_{Fe'L}^{cond}$; $K_{Fe'L}^{cond} = 10^{11.8}$ measured in Aquil at pH 8; Maldonado et al. 2005). Stock DFB solutions (0.1–10 mmol L^{-1}) were prepared as previously described (Maldonado and Price 2001).

Iron-replete cultures of *T. oceanica*, *T. weissflogii*, and *T. pseudonana* were grown at 18° ± 1°C under a continuous PFD of 90 μmol quanta m^{-2} s^{-1} in 10 μmol L^{-1} EDTA-buffered Aquil medium containing 58 nmol L^{-1} total Fe.

We also examined the ability of three Southern Ocean strains (*P. antarctica* [clone AA1], *E. antarctica*, and *P. inermis*), and three temperate diatoms (*Thalassiosira* spp.) to grow on Fe complexed to three additional model ligands, the siderophores ferrichrome, enterobactin, and aerobactin. All siderophores used are hexadentate, the optimal denticity to satisfy the six coordination sites of Fe(III). However, they differ in their functional groups, provenance, and photoreactivity. Ferrichrome and DFB are terrestrial trihydroxymate siderophores that form photostable Fe complexes (Barbeau et al. 2003; Maldonado et al. 2005). Aerobactin, produced by the marine bacterium *Vibrio* sp., possesses one α-hydroxycarboxylate and two hydroxymate functional groups and is photoreactive (Küpper et al. 2006). Enterobactin is a tris-catechol siderophore produced by terrestrial bacteria that forms photostabile Fe complexes (Raymond et al. 2003). The conditional stability constants ($K_{Fe'L}^{cond}$) of the siderophores fall within the range measured for natural ligands in seawater (10^9–10^{13}; Witter et al. 2000): DFB = $10^{11.8}$ (Maldonado et al. 2005), ferrichrome = $10^{12.9}$ (Witter et al. 2000), aerobactin = $10^{12.5}$ (Harris et al. 1979), and enterobactin = $10^{10.8}$ (Witter et al. 2000).

Iron-free ferrichrome (Sigma-Aldrich) and Fe-free aerobactin and enterobactin (EMC Microcollections) were used without further purification. Primary stocks of enterobactin and aerobactin were prepared in methanol and stored frozen. Secondary stocks of all siderophores were prepared in Milli-Q water, filter-sterilized and stored in the dark at 4°C. They were precomplexed with $FeCl_3$ before use and

equilibrated in growth media as described for DFB (Maldonado and Price 2001).

Intracellular Fe and C concentrations—To measure intracellular Fe and C concentrations, cultures were incubated with ^{14}C-bicarbonate (specific activity ~ 28 kBq; PerkinElmer) and $^{55}FeCl_3$ (specific activity 920–2015 MBq L^{-1}; PerkinElmer). Phytoplankton completed at least eight cell divisions before harvesting to ensure uniform labeling. Cultures in mid-exponential phase were filtered by gravity (flow rates < 10 mL min^{-1}) onto either 1.0-μm or 3.0-μm porosity polycarbonate membrane filters (Poretics®). Filters were exposed to Ti(III) EDTA-citrate reagent (3–5 min) and rinsed with chelexed seawater following Maldonado and Price (1996). The ^{55}Fe and ^{14}C activities were measured by liquid scintillation counting (Beckman LS 6500 scintillation counter). Samples were corrected for filter absorption using radiotracer medium without cells. Total intracellular Fe and C were calculated using the specific activities of ^{55}Fe and ^{14}C in the medium and the particulate ^{55}Fe and ^{14}C, correcting for quenching and decay.

Cell size (fL $cell^{-1}$; fL = femtoliter = 10^{-15} L) and cell density (cells mL^{-1}) were determined by Coulter Counter® (Model ZM) and microscopy (Palmer Mahoney chamber). A minimum of 500 cells was enumerated and 50–100 cells were sized for each sample. Each sample was counted three times. *Phaeocystis* and the temperate *Thalassiosira* diatom species were counted and sized by both microscopy and Coulter Counter; no significant difference between methods was observed. Coulter Counter measurements were performed on freshly harvested cultures. Microscopy counts were conducted at the time of harvest or within 1 week of preservation with 0.4% glutaraldehyde. Cell dimensions were always measured in freshly harvested cultures using a calibrated ocular micrometer. Cell volumes and surface areas were calculated assuming the following geometric approximations: *Phaeocystis*—sphere; *F. kerguelensis*—elliptic prism; *E. antarctica*—cube; *P. inermis* and *Thalassiosira* spp.—cylinder.

Short-term Fe uptake experiments—Three short-term Fe uptake experiments were performed with *P. antarctica* (clone AA1) using ^{55}Fe. Mid-exponential phase cultures were concentrated by centrifugation (3000 × *g* for 20 min at 3°C) and resuspended in the organically bound ^{55}Fe uptake media described below. Uptake media were prepared in synthetic ocean water (SOW; Aquil without EDTA, nitrate, trace metal, and vitamin additions). Subsamples of resuspended culture were filtered (0.8 μm polycarbonate) in duplicate four times over 8–15 h to determine the incorporation rate of the ^{55}Fe label (Maldonado and Price 2001). In the first experiment, triplicate cultures grown in Fe-replete (4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA) and Fe-limiting (4 nmol L^{-1} Fe, 400 nmol L^{-1} DFB) media were resuspended in uptake media containing 1000 nmol L^{-1} of DFB and five different Fe concentrations: 4, 10, 100, 500, and 1000 nmol L^{-1}. These additions resulted in [Fe′] of 6, 16, 176, 1585, and 31,700 fmol L^{-1}, respectively. The second experiment was identical to the first except that [Fe′] was manipulated by keeping the Fe concentration constant (4 nmol L^{-1}) and varying the concentration of DFB: 400, 40, and 4 nmol L^{-1}, resulting in [Fe′] of 16, 176, and 31,700 fmol L^{-1}, respectively. In the 4 : 4 and 1000 : 1000 Fe : DFB uptake media, Fe concentrations were reduced to 3.8 nmol L^{-1} and DFB concentrations increased to 1050 nmol L^{-1}, respectively, to ensure that DFB was in excess of ^{55}Fe. In the third experiment, triplicate cultures were preacclimated to Fe-replete medium (4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA), and media containing Fe : DFB of 4 : 4 nmol L^{-1} (Fe-replete growth rates) and 4 : 400 nmol L^{-1} (Fe-limited). Resuspended cultures were then transferred to uptake media containing the ^{55}Fe bound to model siderophores (aerobactin, enterobactin, ferrichrome, and DFB) at a ratio of 4 : 40 nmol L^{-1} or in a medium where 4 nmol L^{-1} of ^{55}Fe was added as Fe : EDTA (1 : 1.05) to prevent Fe precipitation. All short-term Fe uptake incubations were performed at 3°C in the dark, except in one experiment where Fe-limited cultures were incubated in Fe : DFB 100 : 1000 uptake medium in both the dark and under growth irradiance (90 μmol quanta m^{-2} s^{-1}).

Reduction rates of organically complexed Fe(III)—Extracellular reduction of Fe(III) was determined by directly measuring Fe(II) production, essentially as described by Maldonado and Price (2000, 2001). Briefly, duplicate cultures of *P. antarctica* (clone AA1) were grown to mid-exponential phase in Fe-replete (4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA) and Fe-limiting (4 nmol L^{-1} Fe, 400 nmol L^{-1} DFB) media and concentrated by centrifugation. The concentrate was gently filtered onto an acid-washed 2-μm 47-mm polycarbonate filter. The filter was placed in-line on a Fe(II) flow-injection analysis instrument and exposed to pH 6.6 SOW (buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), no Fe added, ~ 2 nmol L^{-1} Fe(III) contamination) and Fe(III)DFB solutions (4 : 1000 to 1000 : 1050 nmol L^{-1} prepared in pH 6.6 SOW). Fe(II) was also measured in the solutions without cells to correct for abiotic Fe(III) reduction. The incubations were performed in the dark at 3°C. Cellular reduction rates were calculated from the steady-state Fe(II) concentration, the flow rate in the sample line (1.15 mL min^{-1}), and the number of cells on the filter. Iron(III) reduction by heat-shocked cells (60°C for 15 min) was < 3% of untreated cells.

Data analysis—Sample means and standard errors were calculated using Microsoft Excel software (version 2004 for Macintosh; Microsoft). Data were examined for normality and equal variance prior to Analysis of Variance (ANOVA) to determine treatment effects. Significant results are reported at the 95% confidence level ($p < 0.05$).

Results

Fe chemistry and phytoplankton growth rates—The effect of Fe chemistry on the steady-state growth rates (μ) of five Southern Ocean isolates is presented in Table 1 and summarizes measurements made from numerous separate

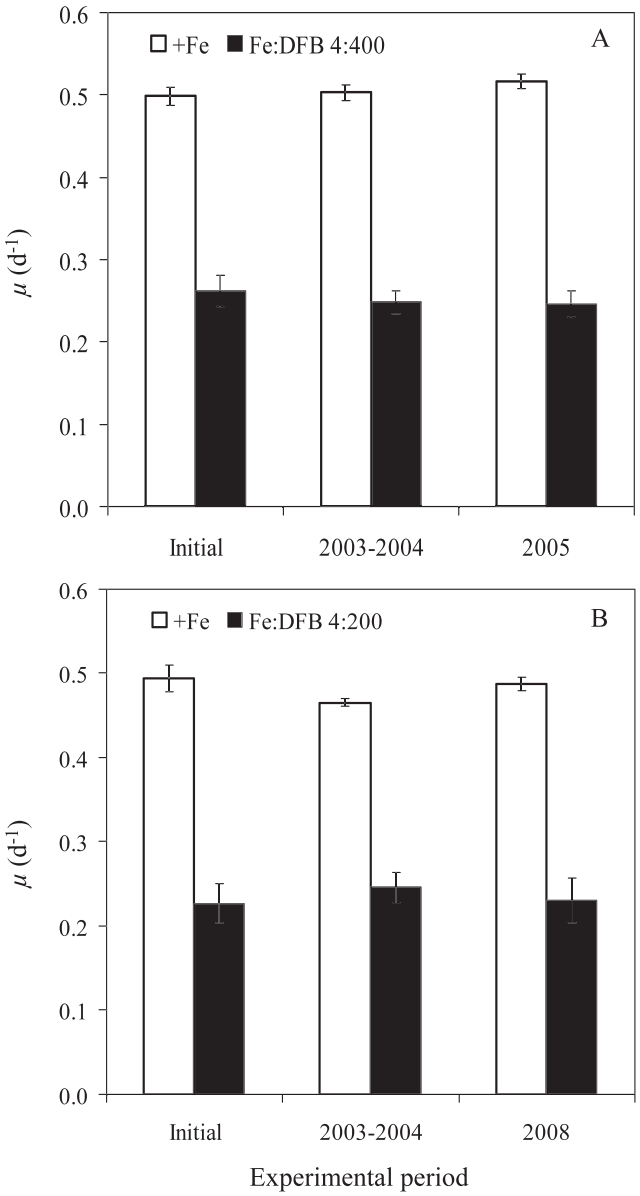

Fig. 1. Growth rates of Southern Ocean isolates measured periodically between 2003 and 2008. 'Initial' rates were measured after preacclimating cultures in growth media for two transfers (14–16 generations) in mid-2003. (A) *Phaeocystis antarctica* (clone AA1) grown in Fe-replete Aquil medium (+Fe) and Fe-limiting medium (Fe : DFB 4 : 400). (B) *Proboscia inermis* grown in Fe-replete Aquil medium (+Fe) and Fe-limiting medium (Fe : DFB 4 : 200). Error bars are standard error (n = 6–27).

experiments conducted over a 5-yr period. The growth rates of Southern Ocean isolates in the experimental media attained steady state within the preacclimation period of two transfers (14–16 generations) and remained stable over the 5-yr period of the study (Fig. 1). In total, 445 independent growth-rate estimates were obtained from fully acclimated cultures in steady state. Maximum growth rates ranged from 0.15 d^{-1} for the large diatom *T. antarctica* (cell volume = 30,000 fL $cell^{-1}$) to 0.53 d^{-1} for solitary cells of the haptophyte *P. antarctica* (clone AA1). The diatom *Proboscia inermis* grew at rates comparable to *P. antarctica* (0.47 d^{-1}) despite being over three orders of magnitude larger (~ 50,000 fL $cell^{-1}$ vs. ~ 20 fL $cell^{-1}$; Table 2). The maximum growth rates of *F. kerguelensis* and *E. antarctica* were 0.32 d^{-1} and 0.36 d^{-1}, respectively. Amongst all of the isolates we examined, maximum growth rates were poorly correlated with cell volume (linear regression, $y = -0.024\text{Ln}(x) + 0.56$, $R^2 = 0.25$, $n = 5$).

With the exception of the diatom *F. kerguelensis*, all of the isolates grew at near maximum rates in 10 μmol L^{-1} EDTA-buffered Aquil media that contained between 1.8 nmol L^{-1} and 4.4 nmol L^{-1} Fe ($\mu : \mu_{max} \geq 0.9$, where μ_{max} is growth rate in Aquil medium with 58 nmol L^{-1} Fe and 10 μmol L^{-1} EDTA). The most dilute Fe medium contained only the Fe that remained as a contaminant following Chelex 100 treatment. Increasing the EDTA concentration in this medium from 10 μmol L^{-1} to 100 μmol L^{-1} did not influence the growth rates of *P. antarctica* or *P. inermis*. Likewise, decreasing Fe contamination from 1.8 nmol L^{-1} to 0.8 nmol L^{-1} (using 8-hydroxyquinonline in place of Chelex 100) had no significant effect on the growth rate of *P. antarctica* or *P. inermis*. The growth rate of *F. kerguelensis* was ~ 50% lower in media containing 1.8–4.4 nmol L^{-1} Fe compared to medium containing 58 nmol L^{-1}. Overall, these results suggest that a sufficiently large fraction of the Fe contamination in Aquil was available to completely fulfill the cellular requirements of four of the five Southern Ocean species we studied.

The complete suite of DFB treatments is presented in Table 1, while the growth of cultures in Fe : DFB media relative to the highest Fe treatment ($\mu : \mu_{max}$) is summarized in Fig. 2. All Southern Ocean isolates, with the exception of *E. antarctica*, grew in medium containing DFB concentrations 100-fold higher than Fe (Fe : DFB 4 : 400 treatment; Fig. 2A,B). *P. antarctica* and *P. inermis* maintained the highest absolute and relative growth rates in Fe : DFB media. Growth rates of these isolates in 4 : 4 and 4 : 40 Fe : DFB media were not significantly different from the highest Fe treatment (ANOVA; $p > 0.05$). Growth rates of *Phaeocystis* and *P. inermis* were reduced by ~ 50% at Fe : DFB ratios of 4 : 400 and 4 : 200, respectively. The growth rates of *E. antarctica*, *T. antarctica*, and *F. kerguelensis* were reduced by ~ 50% at a Fe : DFB ratio of 4 : 40. *Eucampia antarctica* did not grow in Fe : DFB 4 : 400 medium. The growth response of Southern Ocean species in SASW and Fe : DFB media was similar. Isolates that grew at near maximum rates in media containing high concentrations of DFB also grew at near maximum rates in low-Fe SASW: *P. antarctica* was not Fe-limited, *P. inermis* grew at 67% of its maximum rate, and *E. antarctica* did not grow in SASW containing 0.49 nmol L^{-1} Fe and 1.4 nmol L^{-1} natural ligand (Table 1).

The growth in Fe : DFB media of *Thalassiosira* spp. isolated from different oceanic regions is presented in Fig. 2B. The results are presented as relative growth rates ($\mu : \mu_{max}$), because the maximum growth rate of *T. antarctica* was considerably lower (0.12–0.15 d^{-1}) than the maximum growth rates of the temperate species *T. oceanica*, *T. pseudonana*, and *T. weissflogii* (0.81 ± 0.01, 1.1

Table 2. Cell volume-normalized intracellular Fe and C concentrations of Southern Ocean phytoplankton as a function of Fe availability. Units for Fe : DFB (desferrioxamine B) are nmol L^{-1} : nmol L^{-1}. Steady-state Fe uptake rates are normalized to cell surface area (units: nmol Fe m^{-2} d^{-1}). Units for Fe-use efficiency are $\times 10^5$ mol C mol^{-1} Fe d^{-1}. Values within parentheses are standard error (n = 3–9).

Iron treatment*	[Fe′] (pmol L^{-1})	μ (d^{-1})	Cell volume (CV) (fL $cell^{-1}$)	Fe content (μmol L_{CV}^{-1})	C content (mol L_{CV}^{-1})	Fe : C (μmol mol^{-1})	Steady-state Fe uptake	Fe-use efficiency
Phaeocystis antarctica (clone AA1)								
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	258	0.49(0.00)	27.1(1.7)	90.2(3.3)	11.2(0.8)	8.6(0.4)	28.5(1.4)	0.6(0.0)
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.55(0.00)	16.9(1.2)	33.7(3.8)	16.6(1.5)	2.0(0.0)	10.8(1.8)	2.7(0.1)
FeDFB 4 : 4	30.1	0.55(0.02)	18.1(0.7)	39.7(3.5)	16.4(0.9)	2.4(0.1)	12.8(1.3)	2.3(0.1)
FeDFB 4 : 40	0.17	0.53(0.01)	16.7(0.9)	39.9(1.3)	14.5(0.2)	2.7(0.1)	9.1(0.5)	1.6(0.2)
FeDFB 4 : 400	0.02	0.25(0.01)	16.4(0.6)	38.7(1.8)	16.6(0.6)	2.3(0.0)	5.5(0.3)	1.1(0.0)
Phaeocystis antarctica (clone SX9)								
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	258	0.50(0.00)	29.6(2.7)	63.9(5.0)	11.1(2.4)	6.0(0.8)	22.5(2.2)	0.9(0.1)
FeDFB 4 : 400	0.02	0.25(0.00)	16.9(1.7)	31.0(1.8)	15.3(1.4)	2.0(0.2)	4.4(0.2)	1.2(0.1)
Fragilariopsis kerguelensis								
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.18(0.01)	1530(40)	41.0(6.7)	9.9(1.6)	4.2(0.0)	12.0(1.4)	2.2(0.1)
FeDFB 4 : 4	30.1	0.18(0.01)	1180(10)	17.0(1.7)	11.8(0.1)	1.4(0.2)	5.4(0.6)	1.3(0.2)
FeDFB 4 : 40	0.17	0.18(0.01)	1330(20)	11.8(2.0)	14.0(1.8)	0.8(0.0)	3.5(0.5)	2.2(0.1)
Eucampia antarctica								
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	258	0.33(0.01)	15,240(750)	9.5(0.9)	5.3(0.7)	1.8(0.1)	11.1(0.8)	1.9(0.2)
FeDFB 4 : 40	0.17	0.20(0.02)	24,000(1500)	1.4(0.1)	1.5(0.1)	0.9(0.1)	1.3(0.1)	2.2(0.1)
Proboscia inermis								
4.4 nmol L^{-1} 10 μmol L^{-1} EDTA	258	0.51(0.02)	43,800(1100)	5.0(0.3)	1.1(0.1)	4.5(0.3)	7.9(0.6)	1.1(0.2)
2.4 nmol L^{-1} 10 μmol L^{-1} EDTA	140	0.46(0.01)	45,600(1200)	2.9(0.0)	1.0(0.0)	2.8(0.0)	2.5(0.1)	1.6(0.2)
FeDFB 4 : 4	30.1	0.42(0.01)	53,000(300)	1.3(0.1)	0.7(0.1)	1.9(0.1)	2.4(0.3)	2.2(0.1)
FeDFB 4 : 40	0.17	0.36(0.01)	53,200(100)	0.4(0.1)	0.7(0.1)	0.6(0.0)	0.6(0.1)	6.2(0.2)
FeDFB 4 : 200	0.03	0.31(0.03)	53,500(200)	0.2(0.0)	0.5(0.1)	0.4(0.1)	0.3(0.1)	8.2(0.3)

* EDTA = ethylenediaminetetraacetic acid.

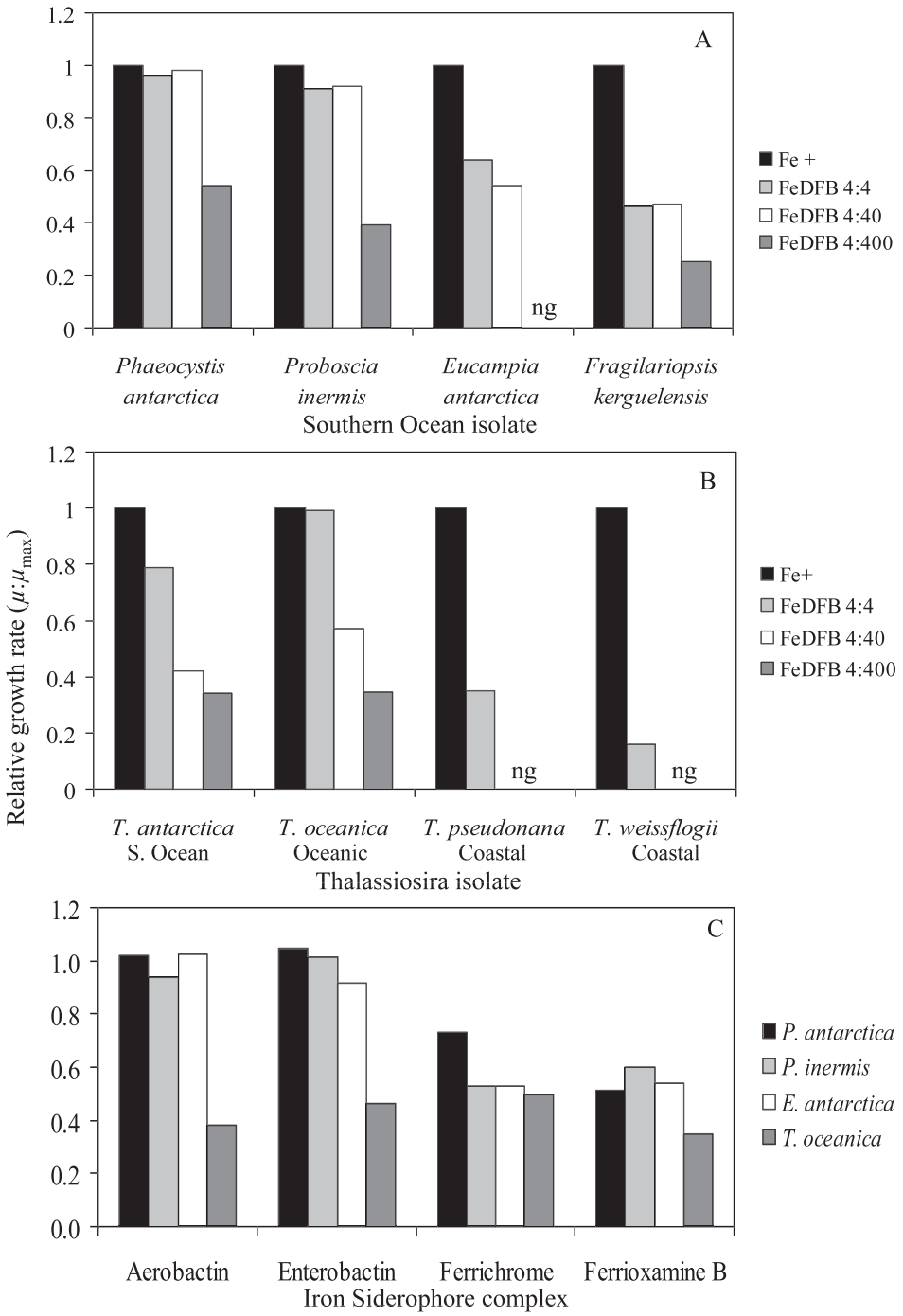

Fig. 2. Growth rates of eukaryotic phytoplankton in Fe-siderophore media (μ) relative to Fe-replete conditions (μ_{max}). (A) Southern (S.) Ocean isolates grown in Fe : DFB media (nmol L^{-1} : nmol L^{-1}) relative to Fe-replete Aquil medium containing 4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA (+Fe). (B) Relative growth rates of *Thalassiosira* isolates from oceanic and coastal habitats. The temperate isolates were grown in Fe-sufficient medium containing 58 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA (+Fe). (C) Relative growth rates of four oceanic isolates on different Fe-siderophore complexes. Media contained 4 nmol L^{-1} Fe complexed to 40 (*Eucampia antarctica*), 200 (*Proboscia inermis*), or 400 nmol L^{-1} siderophore (*Phaeocystis antarctica* and *Thalassiosira oceanica*). These ratios were required to reduce growth by $\sim$ 50% ($\mu:\mu_{max}$ = 0.5) in Fe : DFB media. 'ng' indicates the cultures did not grow. All cultures were grown under continuous PFDs of 80–100 μmol quanta m^{-2} s^{-1}.

$\pm$ 0.1, and 1.0 $\pm$ 0.1 d^{-1}, respectively). Despite the large difference in their absolute growth rates, the two oceanic isolates responded similarly to Fe : DFB treatments. Both isolates grew at $\sim$ 35% of their maximum rate in Fe : DFB 4 : 400 medium and at $\sim$ 50% of their maximum rate in Fe : DFB 4 : 40 medium ($\mu : \mu_{max}$ = 0.42 and 0.57 for *T. antarctica* and *T. oceanica*, respectively). In contrast, repeated attempts to grow the coastal isolates in these media were unsuccessful. In these experiments, we inoculated media with cells that had been acclimated to Fe : DFB 4 : 4 medium. Each incubation resulted in a monotonic decay in chlorophyll fluorescence, which was monitored over 2–3 weeks. However, the coastal species were capable of limited growth on Fe : DFB 4 : 4 medium ($\mu : \mu_{max}$ = 0.35 and 0.16 for *T. pseudonana* and *T. weissflogii*, respectively).

The results of growth experiments using three additional model ligands (ferrichrome, enterobactin, and aerobactin) are shown in Fig. 2C. Here, we employed Fe-siderophore ratios comparable to the Fe : DFB ratios that resulted in a $\geq$ 50% reduction in growth rate. As such, different nanomolar Fe-siderophore ratios were used for different isolates: 4 : 400 for *P. antarctica* (clone AA1) and *T. oceanica*, 4 : 200 for *P. inermis*, and 4 : 40 for *E. antarctica* and the coastal species *T. pseudonana* and *T. weissflogii*. All of the oceanic isolates grew on Fe complexed to these siderophores. The growth rates of the Southern Ocean isolates on Fe complexed to either aerobactin or enterobactin were significantly greater than for FeDFB, and not significantly different from Fe-replete conditions ($p > 0.05$). *Phaeocystis* grew significantly faster on Fe complexed to ferrichrome than FeDFB, whereas growth rates of Southern Ocean diatoms on Fe complexed to ferrichrome were not significantly different from FeDFB ($p > 0.05$). Relative growth rates of *T. oceanica* were 0.35, 0.38, 0.47, and 0.5 for DFB, aerobactin, enterobactin, and ferrichrome, respectively. The coastal species *T. pseudonana* and *T. weissflogii* did not grow in any of the 4 : 40 Fe-siderophore media.

Taxonomic and environmental influences on cellular Fe and C content—The effect of Fe availability on cell volume and elemental composition of five Southern Ocean isolates is presented in Table 2. Iron-limited cells of *P. antarctica* were significantly smaller than Fe-replete cells ($p < 0.05$). In contrast, Fe-limited cells of the diatoms *E. antarctica* and *P. inermis* were significantly larger than Fe-replete cells ($p < 0.05$). The change in cell size was particularly large for *E. antarctica*. Iron-limited cultures of this species were comprised of populations of cells comparable in size to Fe-replete cells and cells that were $\sim$ 10-fold larger. We report the weighted mean of these two populations. The cell size of *F. kerguelensis* did not change systematically with Fe treatment, but we note that all of the treatments presented in Table 2 are Fe-limiting ($\mu : \mu_{max} \sim 0.5$).

The *Phaeocystis* strains used in this study differed morphologically. Iron-replete cultures of clone SX9 were a numerically equal mix of colonial and solitary cells. No colonies were observed in Fe-limited cultures of either strain, or in Fe-replete cultures of clone AA1. Although in the experiments described here, Fe-replete cultures of clone AA1 were composed solely of solitary cells, we note anecdotally that when clone AA1 was still capable of forming colonies, it only did so in Fe-replete medium.

Although clone SX9 formed colonies under Fe-replete conditions, whereas clone AA1 did not, individual cells of the two *Phaeocystis* strains were nearly identical in cell volume and C content ($p > 0.05$). However, intracellular Fe concentrations of both Fe-replete and Fe-limited cultures were significantly lower in clone SX9 ($p < 0.05$). Consequently, clone SX9 had significantly lower Fe : C ratios, lower steady-state Fe uptake rates, and higher Fe-use efficiencies than clone AA1. Steady-state Fe uptake rates were calculated from the product of growth rate (μ) and cell surface-area–normalized intracellular Fe concentrations (Q; viz. Fe uptake = μQ). Similarly, Fe-use efficiency—the rate of C assimilation per unit of (intra)cellular Fe—was calculated by dividing specific growth rate (μ) by the intracellular Fe : C ratio ($\times 10^5$ mol C mol^{-1} Fe d^{-1}).

Cell-volume–normalized intracellular Fe concentrations of Fe-replete cultures ranged from 90.2 μmol Fe L_{CV}^{-1} for *P. antarctica* (clone AA1) to 5.0 μmol Fe L_{CV}^{-1} for the diatom *P. inermis*. In general, the intracellular Fe concentrations of Southern Ocean species decreased progressively with decreasing Fe availability. The one exception to this trend was for *P. antarctica* (clone AA1), in which intracellular Fe concentrations remained relatively constant among the four lowest Fe treatments (33.7–39.9 μmol Fe L_{CV}^{-1}). Cell-volume–normalized C concentrations of cultures grown in high Fe medium ([Fe′] = 450 pmol L^{-1}) ranged from 11.2 mol C L_{CV}^{-1} for *P. antarctica* (clone AA1) to 1.1 mol C L_{CV}^{-1} for the diatom *P. inermis*. Changes in cellular C concentration with decreasing Fe availability were species-specific: cellular C concentration increased $\sim$ 1.4-fold in *P. antarctica* (both clones) and *F. kerguelensis*, and decreased 3.5-fold and 2.2-fold for *E. antarctica* and *P. inermis*, respectively.

We observed that larger species of Southern Ocean phytoplankton required substantially lower intracellular Fe concentrations to support $\sim$ 50% of their Fe-replete growth rates ($\mu : \mu_{max} \sim 0.5$) compared to smaller species (Table 2). This was the case irrespective of whether Fe content was normalized to cell volume or C content. These trends are summarized in Fig. 3. Cell-volume–normalized Fe concentrations of Fe-limited cultures were 38.7 μmol Fe L_{CV}^{-1} for *P. antarctica* (clone AA1), 31.0 μmol Fe L_{CV}^{-1} for *P. antarctica* (clone SX9), 11.8 μmol Fe L_{CV}^{-1} for *F. kerguelensis*, 1.4 μmol Fe L_{CV}^{-1} for *E. antarctica*, and a remarkably low 0.2 μmol Fe L_{CV}^{-1} for the largest diatom we examined, *P. inermis*. Similarly, the Fe : C ratios of Fe-limited Southern Ocean species decreased progressively with increasing cell size: 2.3 μmol Fe mol^{-1} C for *P. antarctica* (clone AA1), 2.0 μmol mol^{-1} for *P. antarctica* (clone SX9), 0.8 μmol mol^{-1} for *F. kerguelensis*, 0.9 μmol mol^{-1} for *E. antarctica*, and 0.4 μmol mol^{-1} for *P. inermis*.

As a consequence of decreased Fe content, calculated steady-state uptake rates normalized to cell surface area decreased with decreasing Fe availability in all species and were lower in larger species (Table 2). Iron-use efficiencies

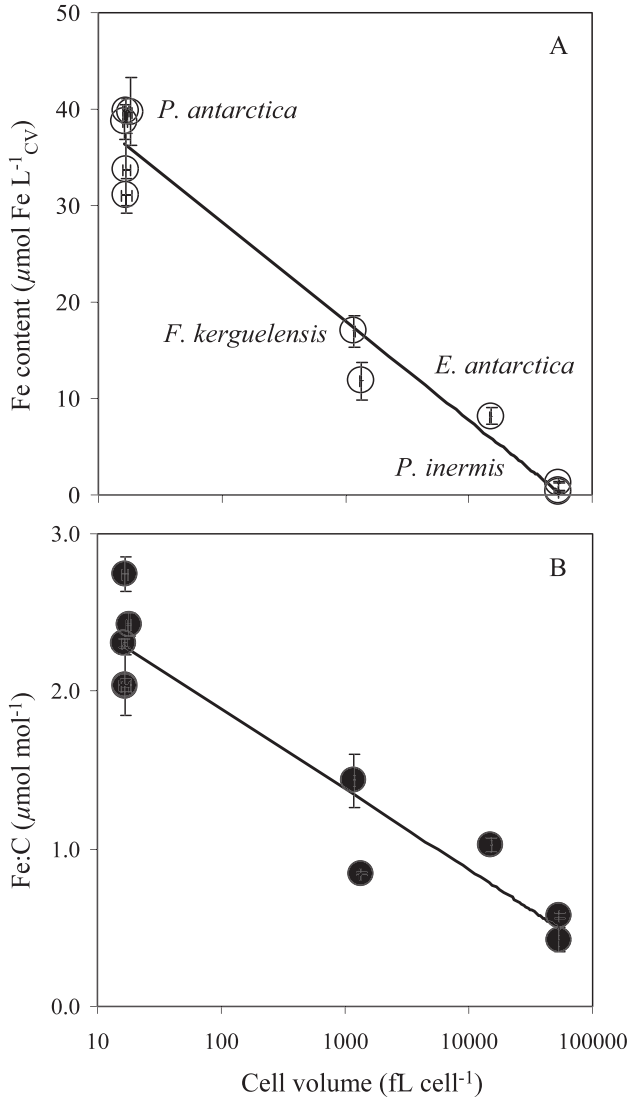

Fig. 3. Relationship between cell volume and the Fe content of 4 Fe-limited Southern Ocean phytoplankton isolates. (A) Cell volume-normalized intracellular Fe content as a function of cell volume. Line indicates regression, $y = -4.46\ln(x) + 48.82$ ($R^2 = 0.97$, $n = 11$). (B) Fe : C ratio as a function of cell volume. Line indicates regression, $y = -0.22\ln(x) + 2.91$ ($R^2 = 0.89$, $n = 11$).

($\times 10^5$ mol C mol^{-1} Fe d^{-1}) were comparable amongst *P. antarctica* (0.6–2.7), *F. kerguelensis* (1.3–2.2), and *E. antarctica* (1.9–2.2). In these species, Fe-use efficiencies remained relatively constant with Fe availability. In contrast, the Fe-use efficiency of *P. inermis* increased from 1.1×10^5 mol C mol^{-1} Fe d^{-1} in Fe-replete cells to 8.2×10^5 mol C mol^{-1} Fe d^{-1} in Fe-limited cells, reflecting the high growth rate maintained by this species (0.31 d^{-1}) despite its remarkably low Fe : C ratio (0.4 μmol Fe mol^{-1} C).

Short-term Fe uptake and Fe(III) reduction—The effects of Fe limitation on the short-term (12–15 h) uptake rates of Fe bound to DFB by *Phaeocystis antarctica* (clone AA1) are presented in Table 3. In this experiment, uptake rates were normalized to cell volume to facilitate direct comparison with the calculated diffusive flux of Fe′. Iron uptake rates were significantly higher ($p < 0.05$) in Fe-limited cultures compared to Fe-replete cultures (Table 3; Fig. 4A). In the lowest Fe treatment, Fe uptake rates of Fe-limited cultures were three-fold higher than for Fe-replete cultures. At higher Fe concentrations, the difference in Fe uptake rates between Fe-limited and Fe-replete cultures was more pronounced: Fe uptake rates were up to 49 times faster in Fe-limited cultures relative to Fe-replete cultures. Iron uptake by Fe-limited cells increased 136-fold (from 0.23 μmol Fe L_{CV}^{-1} h^{-1} to 30.98 μmol Fe L_{CV}^{-1} h^{-1}) following a 250-fold increase in total Fe concentration (from 4 nmol L^{-1} to 1000 nmol L^{-1}). Over this range, Fe′ increased > 5,000-fold (Table 4). In contrast, Fe uptake by Fe-replete cells increased 8.8-fold over this range (from 0.07 μmol Fe L_{CV}^{-1} h^{-1} to 0.63 μmol Fe L_{CV}^{-1} h^{-1}). Rates of Fe uptake from FeDFB were considerably faster than those expected based on the rates of diffusive flux of Fe′ (Table 3). In the 4 : 1000 and 10 : 10000 Fe : DFB uptake media, uptake rates exceeded diffusive flux by 4–12-fold in Fe-replete cultures. In Fe-limited cultures, rates of Fe uptake exceeded diffusive flux, by 5–24-fold, in all uptake media but for the 1000 : 1050 treatment, where uptake rates were ~ 60% of the diffusive flux.

To establish the relationship between Fe uptake from FeDFB and its chemical speciation, an experiment was conducted in which [Fe′] was manipulated by varying total

Table 3. Short-term cell volume – normalized uptake rates of Fe : DFB (desferrioxamine B) by *Phaeocystis antarctica* (clone AA1). Iron-replete cultures were grown in Aquil medium containing 4.4 nmol L^{-1} Fe and 10 μmol L^{-1} EDTA ([Fe′] = 430 pmol L^{-1}). Iron-limited cultures were grown in Aquil medium containing 4 nmol L^{-1} Fe and 400 nmol L^{-1} DFB ([Fe′] = 0.02 pmol L^{-1}). Values within parentheses are standard error ($n = 3$).

Treatment Fe : DFB (nmol nmol L^{-1})	Iron uptake (μmol Fe L_{CV}^{-1} h^{-1})				Diffusive flux of Fe′* (μmol Fe L_{CV}^{-1} h^{-1})		Uptake : flux	
	Iron-replete		Iron-limited		Iron-replete	Iron-limited	Iron-replete	Iron-limited
4 : 1000	0.07	(0.00)	0.23	(0.03)	0.01	0.01	11.5	23.6
10 : 1000	0.07	(0.01)	0.35	(0.10)	0.02	0.03	4.1	13.8
100 : 1000	0.05	(0.00)	1.74	(0.20)	0.18	0.28	0.3	6.2
500 : 1000	0.29	(0.03)	12.63	(0.64)	1.63	2.52	0.2	5.0
1000 : 1050	0.63	(0.08)	30.98	(0.78)	32.52	50.40	0.0	0.6

* The maximum diffusion rate was calculated using the equation $4\pi D[Fe']$, where D is the molecular diffusion coefficient of Fe′ at 0°C: $D_{Fe'} = 4.1 \times 10^{-8}$ dm^2 s^{-1} (Timmermans et al. 2001). Iron-replete cells: cell radius (r) = 1.5 μm, cell volume = 27.1 fL cell^{-1}. Iron-limited cells: cell radius = 1.4 μm, cell volume = 16.4 fL cell^{-1}.

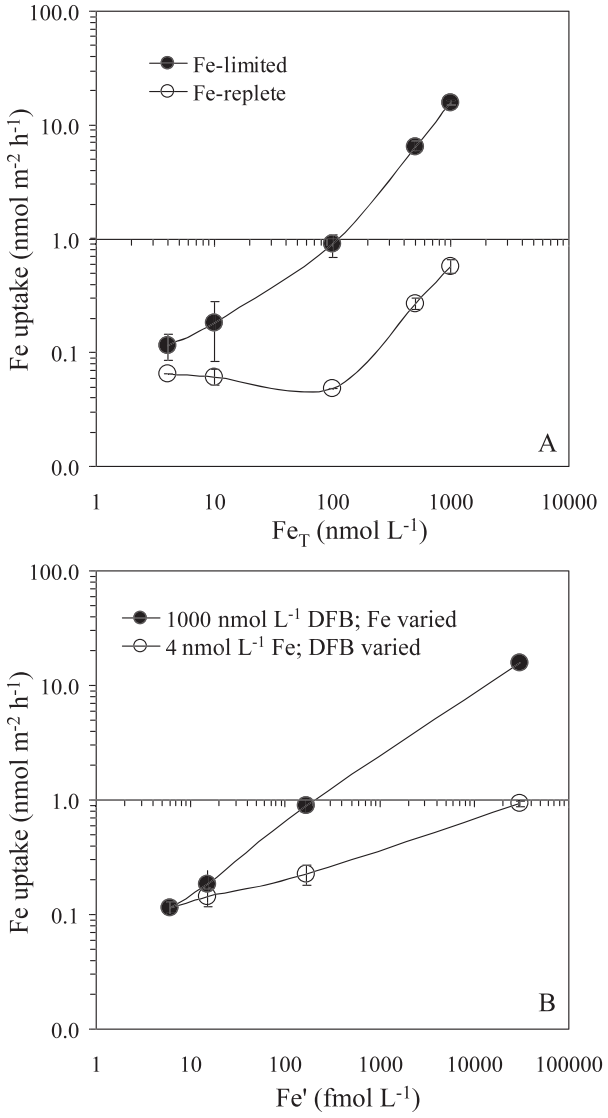

Fig. 4. Short-term cell surface area-normalized rates of Fe uptake from Fe : DFB by *Phaeocystis antarctica* (clone AA1). (A) Fe-replete and Fe-limited uptake rates over a range of Fe concentration (Fe_T) and a constant DFB concentration of 1000 nmol L^{-1}. (B) Uptake rates of Fe-limited cells as a function of Fe′ concentrations. Fe′ was manipulated by changing either Fe or DFB concentrations.

Fe and DFB concentrations. The results of this experiment are presented in Table 4 and the trends summarized in Fig. 4B. In general, the surface-area–normalized rates of Fe uptake of Fe-limited *Phaeocystis* cultures were more responsive to total Fe than to Fe′ concentrations. When [Fe′] was varied by over 5000-fold by keeping the total Fe concentration constant (4 nmol L^{-1}) and varying DFB concentrations (4–1000 nmol L^{-1}) uptake rates varied by eight-fold, from 0.12 ± 0.01 nmol Fe m^{-2} h^{-1} to 0.92 ± 0.06 nmol Fe m^{-2} h^{-1}. However, when the same range of [Fe′] was achieved by keeping total DFB concentrations constant (1000 nmol L^{-1}) and varying Fe concentrations (4–1000 nmol L^{-1}), uptake rates varied over 130-fold, from 0.12 ± 0.01 nmol Fe m^{-2} h^{-1} to 15.7 ± 0.13 nmol Fe m^{-2} h^{-1}. Overall, these results suggest that *Phaeocystis* largely acquires Fe directly from FeDFB. In one experiment, rates of Fe uptake from FeDFB were measured both in the dark and at growth irradiance (90 μmol quanta m^{-2} s^{-1}). Rates of Fe uptake by Fe-limited cultures of *Phaeocystis* incubated in 100 : 1000 Fe : DFB uptake medium were 1.1 ± 0.1 nmol Fe m^{-2} h^{-1} in the dark and 2.4 ± 0.2 nmol Fe m^{-2} h^{-1} under growth irradiance.

One possible mechanism by which *Phaeocystis* could acquire Fe from FeDFB is via extracellular reduction of the DFB-bound ferric ion to the ferrous state by a cell-surface reductase (Maldonado and Price 2000, 2001). We examined this possibility by measuring Fe(II) production by *Phaeocystis* cultures. The experimental results clearly show that *Phaeocystis* reduces Fe(III) bound to DFB to Fe(II; Table 5). Extracellular Fe(II) production was detectable in both Fe-replete and Fe-limited cultures. However, Fe(III) reduction, like FeDFB uptake, appeared to be up-regulated under Fe-limiting conditions: rates of reduction were, on average, 15-fold higher in Fe-limited cells compared to Fe-replete cells (means of all treatments). Reduction rates increased with increasing Fe concentration in the uptake media, from 1.04 ± 0.16 nmol Fe m^{-2} h^{-1} to 5.15 ± 0.77 nmol Fe m^{-2} h^{-1} in Fe-replete cultures and from 12.3 ± 0.6 nmol Fe m^{-2} h^{-1} to 44.5 ± 4.0 nmol Fe m^{-2} h^{-1} in Fe-limited cultures. In all cases, Fe(III) reduction rates exceeded Fe uptake rates. This effect was most pronounced in low Fe media, where reduction rates were 26-fold and 107-fold higher than Fe uptake rates in Fe-replete and Fe-limited cultures, respectively. Iron(III) reduction appeared to be an enzymatic process because reduction rates in heat-shocked samples were < 3% of living cells.

The chemical specificity of the *Phaeocystis* Fe-uptake pathway was investigated using three additional siderophores: enterobactin, aerobactin, and ferrichrome (Fig. 5). In general, Fe uptake from the siderophores was significantly greater in Fe-limited cultures (4 : 400 Fe : DFB-grown cultures) than in Fe-replete cultures (4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA; $p < 0.05$). Cultures grown in 4 : 4 Fe : DFB medium acquired Fe from the siderophores at intermediate rates (Fig. 5A). In Fe-limited cells, the uptake rates of Fe complexed to either aerobactin or enterobactin were not significantly different from FeDFB ($p > 0.05$); uptake rates of Fe complexed with ferrichrome were significantly lower than the other siderophores ($p < 0.05$). Cultures grown in 4 : 4 Fe : DFB medium acquired Fe from DFB and ferrichrome at significantly lower rates than from aerobactin and enterobactin ($p < 0.05$). Uptake rates of Fe from Fe-siderophore complexes were 13–24 times lower than from Fe complexed with EDTA (Fig. 5B). Like siderophore-complexed Fe, Fe bound to EDTA (4 : 4.2 nmol nmol L^{-1}) was taken up at significantly higher rates in Fe-limited cultures compared to Fe-replete cultures ($p < 0.05$).

Discussion

Our study suggests that larger Southern Ocean diatom species require higher ambient Fe concentrations to grow at

Table 4. Short-term cell surface area – normalized rates of Fe uptake by Fe-limited cultures of *Phaeocystis antarctica* (clone AA1). Values within parentheses are standard error ($n = 3$).

[Fe : DFB] (nmol nmol^{-1})	Fe : DFB ratio	[Fe′] (fmol L^{-1})	Iron uptake (nmol Fe m^{-2} h^{-1})	
4 : 4	1 : 1	31,700	0.92	(0.06)
4 : 40	1 : 10	176	0.22	(0.05)
4 : 400	1 : 100	16	0.14	(0.03)
4 : 1000	1 : 250	6	0.12	(0.01)
10 : 1000	1 : 100	16	0.18	(0.05)
100 : 1000	1 : 10	176	0.89	(0.11)
1000 : 1050	1 : 1	31,700	15.7	(0.13)

their optimal rates compared to smaller species (such as solitary cells of *P. antarctica*), due to physical constraints on Fe uptake, such as decreased diffusion to cell volume ratios and lower cell surface area-to-volume ratios; however, they have partially compensated for these constraints in several ways. Firstly, they have evolved strategies to increase the flux of bioavailable Fe—for example, the ability to obtain Fe from strong organic complexes (siderophores). Secondly, they have reduced their biochemical requirements for Fe by means that have yet to be determined. Thus, we have identified two distinct adaptations of Southern Ocean phytoplankton to reduce Fe limitation. To the best of our knowledge, this study is the first systematic examination of the cellular Fe requirements of Southern Ocean species, and provides the first evidence for a ferrireductase Fe uptake pathway in *P. antarctica*. Here, we discuss evidence for the ability of Southern Ocean phytoplankton to acquire organically complexed Fe, and then we examine the evidence for their reduced cellular Fe requirements. We conclude this section with a short discussion on the broader implications of our findings on adaptive strategies to modify phytoplankton Fe requirements with respect to Southern Ocean biogeochemistry.

Acquisition of organically complexed Fe by Phaeocystis antarctica—Early laboratory culture experiments suggested that phytoplankton relied on dissolved inorganic Fe species (Fe′) for uptake and growth, supporting the idea that the Fe transporters at the cell surface react exclusively with Fe′ (Hudson and Morel 1990; Sunda and Huntsman 1995). Subsequent studies have demonstrated that some marine diatoms can increase the supply of Fe′ to the cell surface by reducing Fe(III) bound to organic complexes using an inducible extracellular ferric reductase (Soria-Dengg and Horstmann 1995; Maldonado and Price 2000, 2001). Such observations are of particular relevance to Southern Ocean waters, where essentially all of the dissolved Fe is bound by strong organic ligands (Boye et al. 2001) that buffer such low Fe′ concentrations that they should not, in the absence of photo-redox cycling and/or bioreduction, support phytoplankton growth. Prior to this study, direct evidence for a reductive Fe transport in Southern Ocean phytoplankton has been lacking.

Our experiments suggest that the Fe uptake mechanism employed by *P. antarctica* shares many of the fundamental characteristics of the one described for *T. oceanica* (Maldonado and Price 2001). (1) Fe uptake depends on the cellular Fe nutritional status and was up-regulated under Fe limitation (Fig. 4A; Table 3). (2) The Fe uptake mechanism is non–ligand-specific:Fe was acquired from all of the Fe-siderophore complexes we tested (Fig. 5). In the case of DFB, Fe uptake rates greatly exceeded the diffusive flux of equilibrium concentrations of Fe′ (Table 3). (3) Fe uptake appeared to involve an extracellular reduction of Fe(III) to Fe(II; Table 5). Like Fe uptake, Fe(III) reduction was up-regulated under conditions of Fe limitation.

Although we do not have direct evidence of a reductive Fe transport pathway for Southern Ocean diatoms, we note that previous evidence of this pathway in marine phytoplankton has come exclusively from diatom isolates (Soria-Dengg and Horstmann 1995; Maldonado and Price 2000, 2001). Moreover, the growth response to organically complexed Fe among Southern Ocean diatom and *Phaeocystis* cultures was remarkably similar (Fig. 2; Table 1),

Table 5. Short-term cell surface area – normalized rates of Fe uptake and extracellular Fe(II) production by *Phaeocystis antarctica* (clone AA1). Iron-replete cultures were grown in Aquil medium containing 4.4 nmol L^{-1} Fe and 10 μmol L^{-1} EDTA ([Fe′] = 430 pmol L^{-1}). Iron-limited cultures were grown in Aquil medium containing 4 nmol L^{-1} Fe and 400 nmol L^{-1} DFB [Fe′] = 0.02 pmol L^{-1}. Values within parentheses are standard error ($n = 3$) for Fe uptake rates and ranges ($n = 2$) for Fe(II) production rates. DFB = desferrioxamine B.

Treatment Fe : DFB (nmol nmol L^{-1})	Iron uptake (nmol Fe m^{-2} h^{-1})				Extracellular iron(II) production (nmol Fe m^{-2} h^{-1})			
	Iron-replete		Iron-limited		Iron-replete		Iron-limited	
4 : 1000	0.04	(0.00)	0.12	(0.01)	1.04	(0.16)	12.3	(0.6)
10 : 1000	0.04	(0.01)	0.18	(0.05)	0.64	(0.10)	11.8	(4.8)
100 : 1000	0.03	(0.00)	0.89	(0.12)	0.83	(0.13)	16.6	(2.1)
500 : 1000	0.17	(0.01)	6.50	(0.33)	2.00	(0.30)	33.9	(3.8)
1000 : 1050	0.36	(0.04)	15.7	(0.13)	5.15	(0.77)	44.5	(4.0)

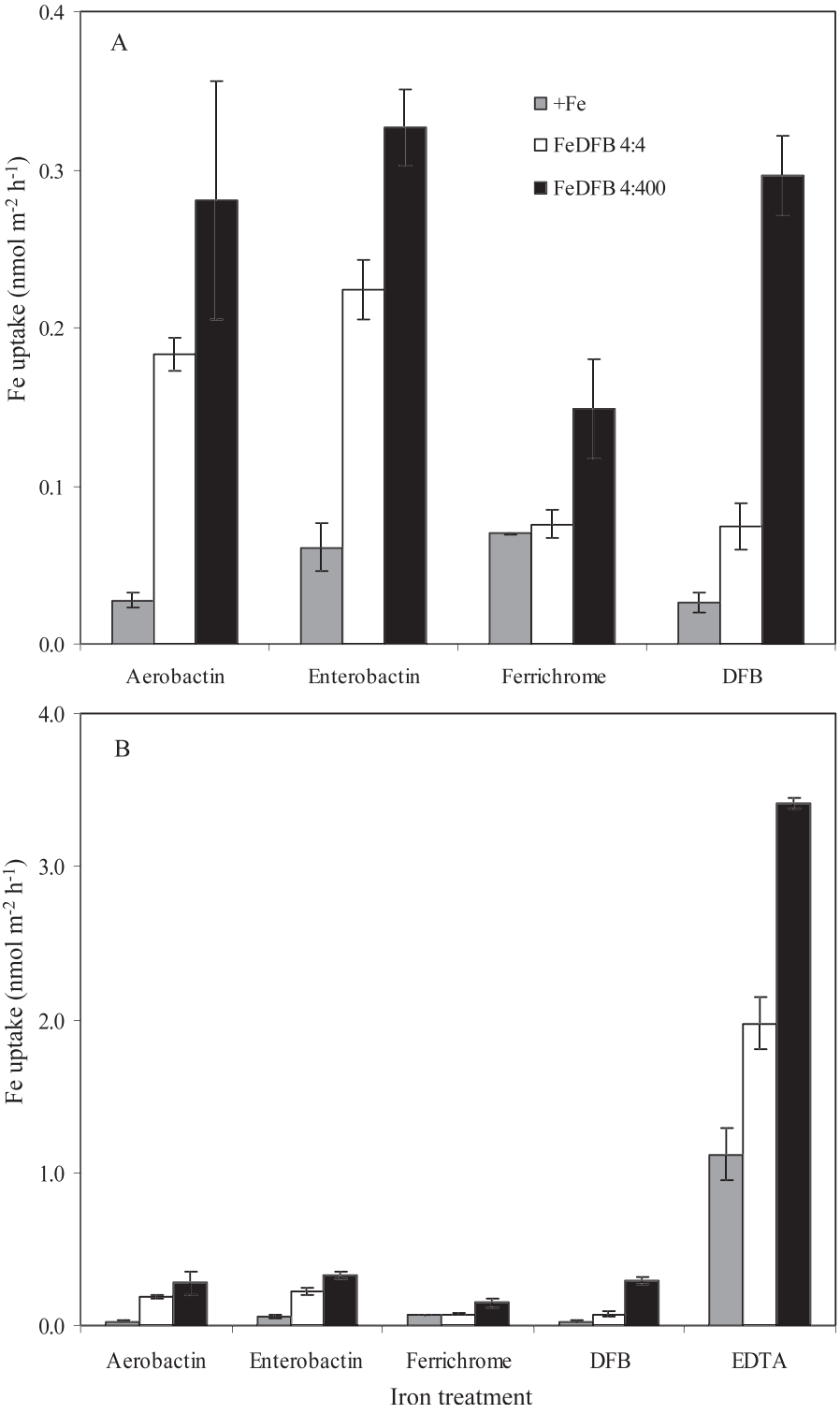

Fig. 5. Short-term cell surface area-normalized rates of Fe uptake from Fe-siderophore complexes by Fe-replete (4.4 nmol L^{-1} Fe, 10 μmol L^{-1} EDTA (+Fe); Fe : DFB 4 : 4) and Fe-limited (Fe : DFB 4 : 400) cultures of *Phaeocystis antarctica* (clone AA1). (A) Fe uptake from hydroxamate (ferrichrome and DFB), catecholate (enterobactin), and mixed functional group (aerobactin) Fe-siderophore complexes (Fe : L = 4 : 40 nmol L^{-1}), and (B) same data as presented in (A) for Fe uptake from Fe-siderophore complexes, but rescaled to include Fe uptake from Fe : EDTA (4 : 4.2 nmol L^{-1}). Error bars are standard error (n = 3).

suggesting strongly that they share a common Fe uptake mechanism.

The dependence of Fe uptake on cellular Fe nutritional state is well-documented. Low Fe concentrations are thought to induce an up-regulation in the number of cell-surface Fe transport systems, resulting in considerably faster Fe uptake rates (Hudson and Morel 1990). In our experiments with *Phaeocystis*, uptake of Fe bound to EDTA increased three-fold in Fe-limited cells compared to Fe-replete cells (Fig. 5B), whereas increases in the uptake of Fe bound to siderophores were more pronounced—up to 11-fold greater in Fe-limited cells (Fig. 5A). Our results thus provide general support for the induction of Fe uptake under conditions of Fe limitation.

The uptake of Fe from FeDFB was up to 44 times faster by Fe-limited *Phaeocystis* cells compared to Fe-replete cells (Fe : DFB 1000 : 1050 medium; Table 5). Remarkably, our results are nearly identical to the 30-fold increase in Fe uptake from FeDFB in Fe-limited cultures of *T. oceanica* under comparable experimental conditions (Fe : DFB 100 : 1000 medium; *T. oceanica* growing at 85% of μ_{max}; *Phaeocystis* growing at 50% of μ_{max}; Maldonado and Price 2001). Although the absolute rates of Fe uptake from FeDFB by Fe-limited *Phaeocystis* were three-fold lower than those measured for *T. oceanica*, we note that the growth rate of *Phaeocystis* was also approximately three-fold lower than *T. oceanica* (0.28 d^{-1} vs. 0.87 d^{-1}). Much of the lower Fe uptake and growth rates for *Phaeocystis* may be due to the lower incubation temperature (3°C) for this species relative to that (18°C) for *T. oceanica*. This result indicates that Fe transport scales with growth and metabolic rates, as would be expected given the dependency of growth rates on the intracellular Fe : C concentrations, which are in turn directly dependent on the cellular Fe uptake rates, in Fe-limited cells (Sunda and Huntsman 1995).

Indeed, the differences between steady-state and short-term Fe uptake experiments provides a means of assessing how factors that affect cell metabolism, such as growth irradiance, affect Fe uptake. Short-term experiments were conducted in the dark over 8–15 h, whereas steady-state measurements were measured in cultures grown under continuous irradiance over several generations. Despite this difference, steady-state and short-term Fe uptake rates for *Phaeocystis* are in good agreement. Cultures grown in 4 : 400 Fe : DFB medium attained steady-state uptake rates of 5.5 ± 0.3 nmol Fe m^{-2} d^{-1} under growth irradiance (Table 2). Short-term Fe uptake by *Phaeocystis* that were preconditioned and exposed to 4 : 400 Fe : DFB medium was 0.14 ± 0.03 nmol Fe m^{-2} h^{-1}, or 3.6 nmol Fe m^{-2} d^{-1} (Table 4) in the dark. Notably, the difference between short-term and steady-state rates can largely be accounted for by irradiance. In a short-term experiment in which Fe uptake from FeDFB was measured in subsamples of *Phaeocystis* incubated in both the dark and at growth irradiance, Fe uptake rates were 2.2-fold higher in the light (2.41 nmol Fe m^{-2} d^{-1} and 1.11 nmol Fe m^{-2} d^{-1} for light- and dark-incubated samples, respectively). This effect would appear to be physiological because the FeDFB complex is nonphotolabile. Maldonado et al. (2005) suggested that prolonged dark incubations negatively affected Fe uptake in natural populations in sub-Antarctic waters. Our results are inconclusive, because the difference in Fe uptake could be due either to the deleterious effects of darkness or due to a stimulation of Fe uptake in the light.

The measured rates of Fe uptake from FeDFB by *P. antarctica* greatly exceeded the calculated diffusive flux rates of Fe′, implying that FeDFB was the substrate for Fe uptake (Table 3). Note that we have used a conservative estimate of the conditional stability constant for DFB ($K_{Fe'L}^{cond} = 10^{11.8}$; Maldonado et al. 2005) compared to previous studies (Maldonado and Price 2000, 2001) that have used the $K_{Fe'L}^{cond}$ value of $10^{16.5}$. The choice of conditional stability constant directly affects the calculation of [Fe′] and, hence, diffusive flux. Despite the roughly four-order-of-magnitude difference in stability constants used, our calculations nonetheless suggest that when Fe concentrations and the Fe : DFB ratio are low, Fe uptake occurs at rates significantly faster (up to 20-fold) than could be supplied by the diffusive flux of equilibrium concentrations of Fe′. Only when Fe concentrations are high and the ratio of Fe : DFB approaches unity are Fe′ concentrations sufficient to satisfy biological demand.

Further evidence that FeDFB is the substrate of Fe uptake was obtained in uptake experiments where Fe′ was manipulated by changing either total Fe or DFB concentrations. Fe uptake was found to have a greater dependence on total dissolved Fe than Fe′ (Fig. 4; Table 4). The decrease in Fe uptake (and growth) at high DFB concentrations that we observed favors the Fe(II) model of Shaked et al. (2005) over the FeL model of Salmon et al. (2006), because the latter model predicts that Fe uptake should remain relatively constant with increasing [L]. According to the Fe(II) model, the mechanism by which growth and uptake are reduced at high ligand concentrations is due not only to the effect of organic ligands on Fe′ concentrations, but also to the competition between ligands on the cell surface (Fe receptors) and ligands in the medium, such as DFB, that bind Fe(II).

In support of the Fe(II) model, we have direct evidence for the production of Fe(II) in *Phaeocystis* cultures (Table 5). As observed for *T. oceanica*, Fe(III) reduction rates exceed Fe uptake rates by approximately an order of magnitude, suggesting that reduction rates are sufficiently high to supply the Fe transporters on the cell surface with Fe derived from organic complexes. The maximum rate of FeDFB reduction we observed for *Phaeocystis* (44.5 nmol Fe m^{-2} h^{-1}; Table 5) is in good agreement with the maximum rate calculated for *T. oceanica* (V_{max} = 57.9 ± 8.2 nmol Fe m^{-2} h^{-1}; fig. 2 of Maldonado and Price [2001]). We note that the reduction rates reported here, measured at pH 6.6 to prevent rapid reoxidation of Fe(II), are merely estimates because (1) rates of Fe(III) reduction tend to increase, and those of oxidation tend to decrease with decreasing pH, and (2) they do not account for the fraction of the Fe(II) produced that was taken up by the cells over the course of the measurements. Nevertheless, our data clearly demonstrate that *Phaeocystis* can reduce, assimilate, and grow on FeDFB.

Growth on organically complexed Fe—One of our principle findings is evidence of the widespread ability to

acquire Fe from organic complexes among Southern Ocean phytoplankton isolates. High concentrations of DFB, which has a much higher affinity for Fe(III) than EDTA ($K_{Fe'L}^{cond} = 10^{11.8}$ and $10^{6.2}$ for DFB and EDTA, respectively; Maldonado et al. 2005) were required to limit the growth of the Southern Ocean isolates (Table 1; Fig. 2A,B). Early reports suggested the Fe bound to DFB was not taken up by marine phytoplankton (Wells 1999) including Southern Ocean diatoms (Timmermans et al. 2001). In contrast, several recent studies have demonstrated that sub-Antarctic and Southern Ocean phytoplankton can take up Fe from FeDFB (Maldonado et al. 2005; Hassler and Schoemann 2009) and grow in its presence (Timmermans et al. 2008). These contradictory results may be due to differences in methodology between studies (e.g., steady-state vs. short-term experiments; laboratory vs. field incubations), the degree of Fe limitation of the experimental organisms (Maldonado and Price 2001), or differences between species in their ability to acquire organically complexed Fe.

Although we observed a marked difference among species of the *Thalassiosira* genus in their ability to grow on Fe complexed by DFB (Fig. 2B), several studies have reported the ability of coastal diatoms to acquire organically complexed Fe. Soria-Dengg and Horstmann (1995) reported uptake of Fe from FeDFB by the coastal diatom, *Phaeodactylum tricornutum*, Hutchins et al. (1999) the uptake of several Fe-siderophore complexes by the coastal diatoms *Skeletonema costatum* and *T. weissflogii*, and Shaked et al. (2005) uptake of Fe from FeDFB by *T. weissflogii*. These findings do not necessarily contradict our own.

Soria-Dengg and Horstmann (1995) employed much higher Fe concentrations in their Fe uptake experiments, ranging from 100 nmol L^{-1} to 4000 nmol L^{-1}, than we used in our growth experiments (4 nmol L^{-1}). As discussed in the previous section, the Southern Ocean isolates we examined likely employ a ferric reductase to access Fe bound to DFB, the same mechanism implicated in the uptake of FeDFB by *P. tricornutum*. As such, the substrate of this reductase (FeDFB) was 25–1000-fold more abundant in the experiments performed by Soria-Dengg and Horstmann (1995) than in the current study. In the uptake experiments of Hutchins et al. (1999) conducted on *T. weissflogii*, Fe concentrations exceeded those used in this study (10 nmol L^{-1} vs. 4 nmol L^{-1}) and an Fe : DFB ratio of 1 : 5 was employed. Notably, our results suggest that *T. weissflogii* grows at a fraction of its maximum rate in Fe : DFB 4 : 4 medium ($\mu : \mu_{max} = 0.16$; Fig. 2B), suggesting that it has a finite ability to acquire organically complexed Fe. However, the growth of *T. weissflogii* is arrested at higher DFB concentrations and/or lower Fe : DFB ratios. Similarly, Shaked et al. (2005) observed that while *T .weissflogii* was able to acquire Fe from FeDFB when it was supplied in a 1 : 1.1 ratio (45 nmol L^{-1} Fe, 49.5 nmol L^{-1} DFB), Fe uptake was significantly inhibited at a Fe : DFB ratio of 1 : 20 (1 μmol L^{-1} DFB).

Methodological differences aside, our results clearly demonstrate that oceanic isolates are better able to fulfill their cellular Fe demand using FeDFB as a substrate compared to coastal species. At present, we do not know if the observed range of abilities to grow on FeDFB and other Fe-siderophore complexes is due to differences between coastal and oceanic species in their Fe acquisition capabilities or their cellular Fe requirements. The latter are known to be greater in coastal species compared to oceanic species (Sunda and Huntsman 1995; Maldonado and Price 1996).

We find it striking, however, that all of the Southern Ocean species we examined can obtain Fe from all of the siderophores we examined, regardless of the provenance (terrestrial vs. marine), Fe-binding groups (catecholate, hydroxamate, and mixed), stability constants, or photolability (aerobactin vs. the other siderophores) of the siderophores we employed. Although the exact nature of the strong ligands in seawater is currently unknown, their chemical characterization has improved dramatically in the last decade. Fe-binding functional groups such as catecholes and hydroxymates have been detected in bulk seawater (Macrellis et al. 2001) and at least a fraction of the hydroxymate ligands are ferrioxamines (Mawji et al. 2008). Furthermore, several reports suggest that Fe bound to other likely constituents of the ligand 'pool,' such as intracellular ligands released into seawater by viral lysis, zooplankton grazing, and bacterial remineralization (Strzepek et al. 2005) is taken up faster than from the siderophores we employed in this study (Hutchins et al. 1999; Hassler and Schoemann 2009).

This leads us to hypothesize, based on our results and those of others, that a significant fraction of organically complexed Fe is potentially bioavailable to eukaryotic phytoplankton in Southern Ocean waters. If this is the case, it would imply that phytoplankton Fe uptake rates may be better correlated with dissolved Fe concentrations than with Fe′ concentrations under some conditions, such as in the dark or at low light intensities, as has been demonstrated empirically for the model oceanic diatom, *T. oceanica*, in laboratory cultures (Maldonado and Price 2001) and suggested by the theoretical arguments of Morel (2008) and the modeling studies of Southern Ocean biogeochemistry of Tagliabue et al. (2008). This may not be the case in the presence of moderate to high irradiance, where the photo-redox cycling of Fe may increase Fe uptake rates by phytoplankton due to its potential to increase Fe′ concentrations (Fan 2008).

From the results of this study, it is not clear what properties of siderophores dictate the bioavailability of Fe. For example, we observed that *Phaeocystis* more rapidly assimilated Fe bound to catecholate siderophores than Fe bound to hydroxamate siderophores (Fig. 5), and that Fe–catecholate complexes supported the highest rates of growth in all of the Southern Ocean isolates we examined (Fig. 2C). However, rates of Fe uptake from aerobactin, enterobactin, and DFB were not statistically different ($p < 0.05$) in Fe-limited *P. antarctica* (Fe : DFB 4 : 400-grown cultures; Fig. 5A), despite a nearly two-order-of-magnitude range in the $K_{Fe'L}^{cond}$ of these siderophores ($10^{10.8}$–$10^{12.9}$), and the fact that these rates were measured in the dark (hence, no photoreduction of Fe-aerobactin). Similarly, *T. oceanica* did not grow faster on enterobactin and aero-

bactin, compared to ferrichrome and DFB, despite the 1–2-order-of-magnitude lower $K_{Fe'L}^{cond}$ of enterbactin ($10^{10.8}$ vs. $10^{12.9}$ and $10^{11.8}$) and the photolability of aerobactin (Fig. 2C). Finally, we observed that *P. antarctica* grew faster on Fe complexed to ferrichrome than to DFB, despite ferrichrome having a $K_{Fe'L}^{cond}$ an order of magnitude greater than DFB ($10^{12.9}$ vs. $10^{11.8}$; Fig. 2C). These results suggest that properties of siderophores other than simply binding strength influence Fe bioavailability.

The photoreactivity of aerobactin was inconsequential to Fe acquisition in our short-term Fe uptake experiments with *P. antarctica*, because these experiments were performed in the dark. The photolability of aerobactin likely played an important role in our culture experiments performed in the light by increasing steady-state concentrations of inorganic Fe(II) ions and Fe(III)′ formed from Fe(II) oxidation (Barbeau et al. 2001). In natural phytoplankton assemblages, photoreduction may play a more prominent role in Fe uptake than in our laboratory experiments (Barbeau et al. 2001; Maldonado et al. 2005). Ascertaining the relative roles of photoreduction and bioreduction will require further characterization of the chemical properties of natural Fe-binding ligands, such as their photoreactivity and the Fe-binding affinity of their potential photoproducts (Barbeau et al. 2001).

Cellular Fe requirements—The Fe use efficiencies of Fe-limited Southern Ocean isolates ($2.8 \pm 2.4 \times 10^5$ mol C mol Fe^{-1} d^{-1}; mean of all Fe-limited treatments, $\mu : \mu_{max} \leq 0.54$, for all species) were not exceptionally high, lying between the average value of 0.66×10^5 mol C mol Fe^{-1} d^{-1} for coastal phytoplankton and 4.6×10^5 mol C mol Fe^{-1} d^{-1} for oceanic isolates (Sunda and Huntsman 1995; Maldonado and Price 1996) except for Fe-limited *P. inermis*, which had Fe use efficiencies comparable to centric diatoms isolated from the Fe-limited Equatorial Pacific (~ 7.2 and 7.0×10^5 mol C mol Fe^{-1} d^{-1}, respectively). However, it is important to note the potential effect that the low temperatures of Southern Ocean waters may have on Fe use efficiencies (which equal the specific growth divided by the cell Fe : C ratio). The results of several studies suggest that water temperatures in the Southern Ocean are suboptimal for the growth of resident populations (Reay et al. 2001; Rose et al. 2009). In effect, low temperature places a metabolic cap on Fe use efficiency. With increasing temperature, we predict that Fe use efficiencies will increase with increasing growth rates, assuming that cellular Fe requirements do not also increase.

Few studies have examined the temperature dependence on cellular Fe requirements, but the available data suggest that Fe : C are relatively invariant over a large temperature range in the coastal diatom *T. weissflogii* (Strzepek and Price 2000). On the other hand, low temperature may have a sparing effect on cellular Fe demands, because low temperature also constrains metabolic rates. Nevertheless, temperature alone cannot explain the exceptionally low Fe requirements of *P. inermis*, because this species maintained comparable absolute growth rates ($\mu = 0.29$–0.31 d^{-1}) but lower Fe : C (0.4 ± 0.1 μmol mol^{-1}) at low temperature (3°C) than the model low-Fe requiring temperate diatom *T. oceanica* grown at 20°C ($\mu = 0.17 \pm 0.09$ d^{-1}; Fe : C = 0.58 ± 0.03 μmol mol^{-1}; Maldonado and Price 1996).

The notable physiological property of the Southern Ocean diatoms was their extremely low intracellular Fe concentrations, whether normalized to cell volume or cell C (Table 2). Steady-state cellular Fe concentrations were negatively correlated with cell volume amongst the Southern Ocean isolates we examined (Fig. 3). The largest species, *P. inermis*, has the lowest Fe : C ratios ever reported (0.4 μmol : mol for cells growing at $\mu : \mu_{max} = 0.6$), although several other oceanic diatom species from the equatorial Pacific and N. Atlantic also have Fe : C ratios $<$ 1 μmol : mol (Maldonado and Price 1996). It is reassuring that comparably low Fe : C ratios for *P. inermis* were found in independent experiments performed under comparable experimental conditions by Lane et al. (2009; 0.7 ± 0.0; this study: 0.6 ± 0.0; Lane et al. (2009) for cells grown in Fe : DFB 4 : 40 medium).

The mean Fe : C ratio for Fe-limited Southern Ocean species in our study was 1.7 ± 1.3 μmol : mol, which agrees favorably with the mean Southern Ocean ratio of 1.8 ± 0.4 μmol : mol ($n = 2$) calculated by Sunda (1997) from regressions of dissolved Fe vs. apparent oxygen utilization. The author predicted that this Fe : C ratio could support a specific growth rate of 0.48 ± 0.1 d^{-1} for *T. oceanica* at 20°C, using the empirically derived relationship: μ (d^{-1}) = $0.268 \times$ Fe : C (μmol mol^{-1}). Despite the lower temperatures used in this study, we found this prediction to be remarkably accurate. However, the largest diatom species we examined (*P. inermis*, cell volume $\sim$ 50,000 fL $cell^{-1}$) was capable of growing at 90% of its Fe-replete rate ($\mu = 0.42$ d^{-1}) with an astonishingly low Fe : C ratio of 0.6 μmol : mol, or roughly twice as fast as predicted by Sunda (1997). This Fe : C ratio is also 2.5 orders of magnitude lower than the optimal Fe : C ratio predicted for the large Southern Ocean diatom *Chaetoceros dichaeta* (cell volume = 75,000 fL $cell^{-1}$) of 200 μmol : mol by Timmermans et al. (2001). Similarly high optimal Fe : C ratios (between 35 and 43 μmol : mol based on 25 cited values) have been suggested by Sarthou et al. (2005) and de Baar et al. (2008). Notably, for these surveys the authors selected published values for Fe-replete diatoms that may have substantial nonmetabolic Fe pools in excess of biological requirements, unlike the present study where we compare the intracellular Fe to C ratios for cells growing at half of their maximum rate. We, therefore, suggest that these 'optimal' ratios are overestimates.

The means by which Southern Ocean diatoms have reduced their cellular Fe demand are currently unknown. Theoretical calculations (Raven 1990) and empirical measurements of model *Thalassiosira* species (Strzepek and Harrison 2004) suggest that the iron–sulfur proteins and cytochromes necessary for photosynthetic electron transport account for the majority of cellular Fe demand. Some oceanic diatom species have lowered their cellular Fe requirements by drastically reducing the abundance of Fe-rich photosynthetic protein complexes (photosystem I and the cytochrome b_6f complex) relative to Fe-poor photosystem II and by replacing Fe-containing cytochrome c_6 with

Table 6. Comparison of Fe : C ratios calculated using a cellular C from biovolume algorithm and Fe : C measured directly using radiotracers.

Iron treatment*	Measured Fe : C	Calculated Fe : C†	Estimate : Actual	Twining et al. 2004
Autotrophic flagellates	—	—	—	8.7(6.5)‡
Phaeocystis antarctica (clone AA1)				
4.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	8.6	9.8	1.1	—
2.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	2.0	1.7	0.8	—
FeDFB 4 : 4	2.4	2.1	0.8	—
FeDFB 4 : 40	2.7	2.7	1.0	—
FeDFB 4 : 400	2.3	2.0	0.9	—
Phaeocystis antarctica (clone SX9)				
4.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	6.0	6.8	1.1	—
FeDFB 4 : 400	2.0	1.9	0.9	—
Mean±SD	—	—	0.96±0.13	—
Diatoms	—	—	—	6.0(4.5)‡
Fragilariopsis kerguelensis				
2.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	4.2	2.5	0.61	—
FeDFB 4 : 4	1.4	0.76	0.53	—
FeDFB 4 : 40	0.8	0.37	0.44	—
Mean±SD	—	—	0.53±0.08	—
Eucampia antarctica				
4.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	1.0	0.50	0.49	—
FeDFB 4 : 40	0.9	2.21	2.38	—
Mean±range	—	—	1.4±1.3	—
Proboscia inermis				
4.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	2.6	4.9	1.9	—
2.4 nmol L^{-1} 10 μmol L^{-1}				
EDTA	1.4	2.7	1.9	—
FeDFB 4 : 4	1.9	10.9	5.7	—
FeDFB 4 : 40	0.6	3.4	6.0	—
FeDFB 4 : 200	0.4	2.4	5.6	—
Mean±SD	—	—	4.2±2.1	—

* DFB = desferrioxamine B; EDTA = ethylenediaminetetraacetic acid.

† We used the Menden-Deuer and Lessard (2000) conversion: Carbon (pg $cell^{-1}$) = 0.288 × cell volume (fL $cell^{-1})^{0.811}$ to recalculate C content from our measured cell volumes. This conversion factor was used by Twining et al. (2004) to estimate the C content of in situ Southern Ocean phytoplankton whose cellular Fe concentrations were assessed using synchrotron-based X-ray fluorescence (SXRF).

‡ Values within parentheses are estimated intracellular Fe : C ratios, assuming that 75% of total cellular Fe concentrations are intracellular (Twining et al. 2004).

copper-containing plastocyanin (Strzepek and Harrison 2004). As photoacclimation affects the relative and absolute abundance of Fe-containing photosynthetic complexes, the effect of light on the cellular Fe demand and photosynthetic architecture of Southern Ocean phytoplankton merits future investigation.

The strong negative correlation between Fe : C molar ratios and cell volume that we observed for Southern Ocean isolates (Fig. 3) is also apparent in previous laboratory studies of temperate coastal and oceanic phytoplankton isolates of Sunda and Huntsman (1995, 1997) and in Southern Ocean natural assemblages (Twining et al. 2004). At first glance, the higher Fe : C ratios measured for natural diatom assemblages in the Southern Ocean during the Southern Ocean Iron Experiment (SOFeX; ~ 6 μmol : mol) are at odds with the Fe : C ratios we report for laboratory cultures. There are a number of factors that can affect the measurement of Fe in the field as identified by Twining et al. (2004). Here we focus on one potential cause for this discrepancy.

The data presented in Table 6 demonstrate that our observed Fe : C ratios can be largely reconciled with Fe : C ratios measured on Southern Ocean phytoplankton by Synchrotron X-Ray Fluorescence (SXRF; Twining et al. 2004). The difference arises from the algorithm of Menden-Deuer and Lessard (2000) used by Twining et al. (2004) to convert cell volume to cellular C concentration. Although this algorithm appears to be robust for small cells (e.g., *Phaeocystis*), it is not so for large cells. Fe : C ratios estimated for *P. inermis* using the algorithm are four-fold higher than those measured directly with radiotracers, due to an underestimation of cellular C. The algorithm also overestimates cellular C concentrations for *F. kerguelensis* by two-fold, and both over- and underestimates cellular C

concentrations for *E. antarctica*, resulting in Fe : C ratios that are two-fold lower or higher than Fe : C ratios measured directly with radiotracers.

Implications of low Fe : C ratios for Southern Ocean biogeochemistry—Given the efforts and focus on improving the accuracy of Fe supply estimates to this region (Moore and Braucher 2008; Boyd and Ellwood 2010), it is equally important to reduce uncertainties in our estimates of the Fe : C ratios of the key phytoplankton groups in the Southern Ocean. Our findings point to the evolution of adaptive strategies to both reduce Fe demand, and to access a wide range of bioavailable Fe in these waters. The presence of such adaptive strategies for Southern Ocean phytoplankton will alter how we simulate Fe demand for this region.

In the past, modelers have had to rely on either long-publicized and highly cited Fe : C ratios, such as from studies on temperate phytoplankton (Sunda and Huntsman 1995), or purely theoretical Fe : C ratios to parameterize modeling studies of Southern Ocean biogeochemistry. We contend that our new data, collected from Southern Ocean isolates, are better suited for this task. For example, although the Fe : C ratios derived from laboratory studies (Sunda and Huntsman 1995) used in the modeling studies of Moore et al. (2002; 1–7 μmol : mol) and Aumont et al. (2003; 2.86 μmol : mol) are in reasonable agreement with the observed ratios of our Fe-limited Southern Ocean isolates growing at half of their Fe-replete rate (0.4–2.3 μmol : mol), they may in some cases overestimate phytoplankton Fe demand by as much as 18-fold. Furthermore, the higher theoretical values of Tagliabue and Arrigo (2005; 10 μmol : mol for diatoms) and Mongin et al. (2006; $\sim$ 80 μmol : mol as the minimum for Fe-replete phytoplankton growth) may greatly overestimate phytoplankton Fe demand, by up to 100-fold, when compared to our lab-culture–based observations. Such an overestimation will have ramifications for the biogeochemical simulations for this region, overestimating phytoplankton Fe demand and, hence, making it difficult to resolve the balance between Fe supply (Moore and Braucher 2008) and demand. Moreover, because the algal Fe : C ratio is pivotal in linking the biogeochemical cycles of Fe and C in the Southern Ocean, an overestimation of Fe demand may result in a consequent underestimation of primary production and C export.

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